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VOLATILE COMPOUNDS FROM ANAL GLANDS OF THE WOLVERINE, *Gulo gulo*

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Abstract—Dichloromethane extracts of wolverine (*Gulo gulo*, Mustelinae, Mustelidae) anal gland secretion were examined by gas chromatographyYmass spectrometry. The secretion composition was complex and variable for the six samples examined: 123 compounds were detected in total, with the number per animal ranging from 45 to 71 compounds. Only six compounds were common to all extracts: 3-methylbutanoic acid, 2-methylbutanoic acid, phenylacetic acid, α-tocopherol, cholesterol, and a compound tentatively identified as 2-methyldecanoic acid. The highly odoriferous thietanes and dithiolanes found in anal gland secretions of some members of the Mustelinae [ferrets, mink, stoats, and weasels (*Mustela* spp.) and zorillas (*Ictonyx* spp.)] were not observed. The composition of the wolverine's anal gland secretion is similar to that of two other members of the Mustelinae, the pine and beech marten (*Martes* spp.).

Key Words V Wolverine, *Gulo gulo*, Mustelinae, Mustelidae, scent marking, fear-defense mechanism, short-chain carboxylic acids.

INTRODUCTION

The wolverine (*Gulo gulo*) is the largest terrestrial member of the Mustelidae and is part of the subfamily, Mustelinae, which includes ferrets, fishers, martens, mink, stoats, weasels, and zorillas. Historically, two species of wolverine have been described, one from North America and the other from

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Eurasia, but today only one species is recognized (Pasitschniak-Arts and Larivière, 1995). The wolverine, as with most mustelids, possesses paired glands that open on both sides of the anus, which produce a tannish-yellow, highly odoriferous secretion (Hash, 1987). Wolverine anal musking has been described by several authors (Haglund, 1966; Pulliainen and Ovaskainen, 1975; Koehler et al., 1980; Magoun, 1985) as a method of scent marking in addition to marking with feces or urine (Jackson, 1961). Others have suggested that anal gland secretions function primarily as a fear-defense mechanism (Krott, 1959; Ewer, 1973; Long, 1987).

The composition of the anal gland secretion from some members of the subfamily Mustelinae was described in a recent review (Burger, 2005). Odoriferous cyclic sulfides (thietanes and dithiolanes) and in some cases, sulfides, disulfides, and other organosulfur compounds, were found in the secretion from smaller members of this subfamily: American mink, *Mustela vison* (Brinck et al., 1978, 1983; Schildknecht et al., 1976, 1981); ferret, *Mustela putorius* (Crump, 1980b; Crump and Moors, 1985; Schildknecht et al., 1976; Schildknecht and Birkner, 1983); Siberian weasel, *Mustela sibirica* (Zhang et al., 2002, 2003); steppe polecat, *Mustela eversmanni* (Zhang et al., 2002, 2003); stoat, *Mustela erminea* (Brinck et al., 1983; Crump, 1978, 1980a; Crump and Moors, 1985; Schildknecht and Birkner, 1983); weasel, *Mustela nivalis* (Brinck et al., 1983; Buglass et al., 1991; Schildknecht and Birkner, 1983) and zorilla, *Ictonyx striatus* (Apps et al., 1988; Wheeler et al., 1997). These sulfur compounds are absent in anal gland secretion of the two other members of the Mustelinae that have been investigated, the pine marten (*Martes martes*) (Brinck et al., 1983; Schildknecht and Birkner, 1983) and the beech marten (*Martes foina*) (Schildknecht and Birkner, 1983). Schildknecht and Birkner (1983) reported short-chain carboxylic acids from these two species, whereas Brinck et al. (1983) identified benzaldehyde from the pine marten. We investigated the anal gland secretion of the wolverine and report the analysis of volatile components in the secretion.

METHODS AND MATERIALS

Anal glands from four individuals (sex not recorded) were obtained incidental to fur trapping during the winter of 2002Y2003 from the Brooks Range in Alaska. These glands were excised from the trapped animals and immediately frozen at −20°C until they could be analyzed. Upon thawing, a 0.25-g sample of the secretion from both glands for each individual was pooled and extracted with 2 ml of CH_2Cl_2. Two additional pooled samples of anal gland secretion from a male and a female were obtained from Glacier National Park, Montana. These samples were kept at −20°C until they were extracted with CH_2Cl_2 as had been done with the previous samples. Control samples of CH_2Cl_2 were analyzed to identify contaminants in the solvent.

Extracts were analyzed by gas chromatographyYmass spectrometry (GC-MS) in splitless mode (0.5 min), using a Hewlett-Packard GCD Plus fitted with a 30 × 0.25-mm HP-5MS column. The GC was programmed from 40°C/4 min, then 30°C/min to 325°C/2 min. Mass spectral fragments below *m*/*z* 39 were not recorded. The relative amount of each component is reported as the percent of the total ion current (TIC). Minor components less than 0.1% of the TIC were not investigated and impurities found in the solvent were subtracted from the analyses.

Compounds were initially identified by comparison of mass spectra to those in the NIST 1998 mass spectral database. All compounds that did not match spectra in the NIST library were examined to see if they matched published spectra of thietanes, dithiolanes, and other organosulfur compounds reported from some members of the Mustelinae (Brinck et al., 1983; Crump, 1980a,b; Crump and Moors, 1985; Schildknecht et al., 1981; Schildknecht and Birkner, 1983; Zhang et al., 2002, 2003). Authentic samples were used to confirm identifications by comparisons of spectra and retention times, except for compounds that were not commercially available or were prepared synthetically (see Table 1). The chirality of compounds containing stereogenic centers was not determined. Reference samples were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and Fisher Scientific (Acros, Pittsburgh, PA, USA), except the following, which were synthesized. Isoamyl laurate was prepared by acid-catalyzed reaction of the corresponding alcohol and carboxylic acid. 3-Methyl-dihydro-2(3*H*)furanone was prepared from 3-methyl-2(5*H*)furanone by catalytic hydrogenation with platinum oxide and H_2. 2-Methylpropanamide and 3-methylbutanamide were prepared from 2-methylpropanoic and 3-methylbutanoic acids by treatment with thionyl chloride to form the acid halides and subsequent reaction with ammonia.

RESULTS AND DISCUSSION

The CH_2Cl_2 extracts of the anal gland secretion from the six wolverines showed great variation between individuals. In all, 123 peaks were seen in the chromatograms, with the number per animal ranging from 45 to 71 (Table 1). We identified 49 of the 123 compounds in the extracts by comparison of their retention times and mass spectra with those of authentic samples. The tentative identification of ten additional compounds could not be confirmed because authentic standards were not available.

The secretions had few of these 59 compounds in common V 26 of these compounds were observed in only one sample. Only six of the identified

TABLE 1. VOLATILE COMPOUNDS IDENTIFIED AND TENTATIVELY IDENTIFIED IN THE ANAL GLAND SECRETION OF WOLVERINE (% TOTAL ION CURRENT)

	Anal gland samples					
Compound	1[a]	2[a]	3[a]	4[a]	5[b]	6[c]
Carboxylic acids						
2-Methylpropanoic acid						7.3
3-Methylbutanoic acid	3.6	2.9	3.2	14.8	1.6	47.2
2-Methylbutanoic acid	0.5	0.7	0.7	1.4	0.2	9.2
Pentanoic acid						0.3
2-Methylpentanoic acid	0.2			1.6		0.3
Hexanoic acid				0.3	0.4	
2-Methylhexanoic acid				0.2		
Heptanoic acid		0.1			0.7	
Octanoic acid	0.3	0.6			3.2	
2-Octenoic acid		0.4				
7-Octenoic acid[d]	0.2					
2-Methyloctanoic acid				3.4		
4-Methyloctanoic acid[d]					0.7	
Phenylacetic acid	0.5	0.2	0.2	2.0	0.9	3.0
Nonanoic acid		0.1			1.1	
3-Phenylpropanoic acid			8			
Decanoic acid		0.1			2.2	
2-Methyldecanoic acid[d]	0.8	2.5	3.4	5.5	3	0.7
2-Decenoic acid		0.7				
Dodecanoic acid				0.2	0.4	
Tetradecanoic acid	1.4	1.4		0.4		
Pentadecanoic acid	2.6	2.4		0.5	2.1	
(*Z*)-9-Hexadecenoic acid	2.5	1.7	0.3		1.9	
Hexadecanoic acid	5.9	6	1	1.3	5	
Linoleic acid	15.5	12.2	3.3	5.2	10.5	
Oleic acid	0.1				4.3	
Stearic acid	3.4	4.5	1	1.6	0.3	
Alcohols						
3-Methyl-1-butanol	0.1					0.4
1-Octen-3-ol			0.2			
Benzyl alcohol		0.1			0.1	1.0
Linalool	0.1		0.6			
2,3-Dimethylcyclohexanol[d]		0.1				0.2
2-Phenylethanol			0.1			
1,2-Hexadecanediol[d]	7	9.4	3.3		6.1	3.8
α-Tocopherol	1	1	8.1	11.2	0.9	0.1
Cholesterol	3.2	3.6	11.1	11.5	2.4	3.3
Hydrocarbons						
Nonane		0.2				
α-Pinene					0.1	
β-Pinene					0.1	
3-Methylnonane[d]		0.1				

TABLE 1. CONTINUED

Compound	Anal gland samples					
	1[a]	2[a]	3[a]	4[a]	5[b]	6[c]
Decane		0.8	0.2			
Undecane		0.4				
Nitrogen compounds						
2-Methylpropanamide						0.2
3-Methylbutanamide						0.6
2-Pyrrolidinone						0.8
2,5-Pyrrolidindione						0.8
2-Piperidinone		0.6	0.2	0.4		2.1
Methylpyrrolidinone[d]		0.2				
Indole						0.3
Other compounds						
Dimethyl disulfide	0.1			0.2		0.1
Dimethyl trisulfide	0.2				0.1	0.1
3-Methyl-dihydro-2(3*H*)furanone						0.3
4-Methyl-dihydro-2(3*H*)furanone[d]						1.7
Benzaldehyde					0.2	
3-Octanone			0.1			
2-Pentylfuran[d]	0.1				0.1	
4-Hydroxybenzaldehyde						0.8
Isoamyl laurate		0.1				
5,6,7,7-Tetrahydro-2(4*H*)benzofuranone[d]	0.3	0.4	0.2		0.5	0.4
Subtotal (% TIC)	49.6	53.5	45.2	61.7	49.1	85.0
No. identified compounds	18	23	16	17	22	20
No. tentatively identified compounds	5	6	3	1	5	5
No. unidentified compounds	45	42	37	29	44	21
Subtotal (% TIC)	51.4	46.5	54.8	38.3	51.9	15.0

[a] From Alaska.
[b] Male from Montana.
[c] Female from Montana.
[d] Identified only by NIST Library match.

compounds were common to all extracts: 3-methylbutanoic acid, 2-methylbutanoic acid, phenylacetic acid, 2-methyldecanoic acid (tentative identification only), α-tocopherol, and cholesterol. Hexadecanoic, linoleic, and stearic acids, and two tentatively identified compounds [5,6,7,7-tetrahydro-2(4*H*) benzofuran and 1,2-hexadecanediol] were common to five of the extracts. Only pentadecanoic and (*Z*)-9-hexadecenoic acids were common to four of the six samples.

The odoriferous thietanes or dithiolanes found in some other mustelines (*Mustela* spp.) were not observed in wolverine anal gland secretion. The fetid nature of the secretion is due in part to the short-chain carboxylic acids and phenylacetic acid found in all of the samples. Four of the samples contained small amounts of dimethyl disulfide and/or dimethyl trisulfide, compounds that would also contribute to the odor, and one contained 8.0% of pungent 3-phenylpropanoic acid. All samples contained a large number of relatively nonvolatile components, including free fatty acids and cholesterol. Thus, the chemical composition of the wolverine's anal gland secretion is similar to that of the pine and beech marten (*Martes* spp.). The anal gland secretions from these species were reported to contain short-chain acids and benzaldehyde (Brinck et al., 1983; Schildknecht and Birkner, 1983), but not the cyclic sulfur compounds found in other members of the Mustelinae (ferrets, mink, stoats, weasels, and zorillas). These chemical differences are consistent with recent mitochondrial DNA (Dragoo and Honeycutt, 1997) and nuclear DNA studies (Koepfli and Wayne, 2003) that indicate the wolverine and martens are more closely related to each other than either is to mustelines of the genera *Mustela* or *Ictonyx*.

Mustelinae anal gland composition has been reported as variable, relating to differences in age, sex, and estrous condition (Crump, 1980a,b; Zhang et al., 2002, 2003), and also as relatively stable, not varying for individuals during the year, by sex, or during the reproductive period (Brinck et al., 1978). Similarities in chemical products within species have led to hypotheses regarding species and individual recognition, but the semiochemical function of anal sac secretions remains unclear (Albone, 1984). Future investigations would be benefited by an increased sample of anal gland material from wolverines of known sex, age, and season.

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